alanine bound. From this, we can conclude that one of the two sodium binding sites is fully disrupted in this conformation leading to the release of this ion in almost all of the simulations. Furthermore, in one of the simulations we observe release of alanine along with the second sodium ion, and are thus able to describe the translocation process in atomic detail.

1. *Br J Pharmacol* (2012) **167**, 256-278.
2. *Mol Pharmacol* (2006) **70**, 1630-1642.
3. *Nature* (2012) **481**, 469-474

2094-Pos Board B113

Molecular Dynamics Studies of Homo-Oligomeric Ion-Channels

Thuy Hien Nguyen, Zhiwei Liu, **Preston Moore**.
University of the Sciences in Philadelphia, Philadelphia, PA, USA.

Ion-channels embedded within biological membranes help maintain osmotic equilibrium, facilitate bioenergetics, and provide the means for transmitting environmental signals. For the rational design of new therapeutics, it is imperative to understand ion-channels interactions in the 'interfacial' region between the aqueous phase and the hydrophobic core of the bilayer. using molecular dynamics simulations of synthetic LS2 and LS3 channels ranging from 1-6 peptides, the structural dynamics of alpha-helices in their membrane environment was characterized. Results show that higher order bundles do not remain in a symmetric packing arrangement but rather form lower order bundles that interact with each other. For example, the LS2 channel is most stable as a tetrameric bundle that is composed of a ''dimer of dimers'', while the LS3 channel is most stable as a hexamer comprised of a ''dimer of trimers''. In addition, lipid perturbation was found to be strongest for bundles consisting of three or less peptides, where it was found that there is a strong correlation between the tilt angles of the helix/helices of each system with the hydrophobic lipid mismatch and the lipid orientational distribution. These structural results affect the flux of water through the channel, where LS2 was found to have the a maximul flux of water as a tetrameric structural arrangement while water flux through LS3 was maximul as the hexameric arrangement, in agreement with experiment. By understanding the interactions of ion-channels embedded within the membrane, it provides pivotal information in the design of antimicrobial, antiviral, and pharmaceutical agents that target ion-channels.

2095-Pos Board B114

Reconciling the Roles of Kinetic and Thermodynamic Factors in Membrane-Protein Insertion

James C. Gumbart[1], Ivan Teo[2], Benoit Roux[3], Klaus Schulten[2].
[1]Georgia Tech, Atlanta, IL, USA, [2]UIUC, Urbana, IL, USA, [3]U. Chicago, Chicago, IL, USA.

For the vast majority of membrane proteins, insertion into a membrane is not direct, but rather is catalyzed by a protein-conducting channel, the translocon. This channel provides a lateral exit into the bilayer while simultaneously offering a pathway into the lumen. The determinants of a nascent protein's choice between these two pathways are not comprehensively understood, although both energetic and kinetic factors have been observed. To elucidate the specific roles of some of these factors we have carried out extensive all-atom molecular dynamics simulations of different nascent transmembrane segments embedded in a ribosome-bound bacterial translocon, SecY. Simulations on the microsecond time scale reveal a spontaneous motion of the substrate segment into the membrane or back into the channel, depending on its hydrophobicity, while potential of mean force (PMF) calculations confirm that the observed motion is the result of local free-energy differences between channel and membrane. Based on these, and other, PMFs, the time-dependent probability of membrane insertion is determined and is shown to mimic a two-state partitioning with an apparent free energy that is compressed relative to the molecular-level PMFs. It is concluded that insertion kinetics underlie the apparent thermodynamic partitioning process that is observed experimentally.

2096-Pos Board B115

From the Micelle to the Membrane: Molecular Dynamics Simulations of Solution NMR Structures of Membrane Proteins

Sarah Rouse, Mark S.P. Sansom.
University of Oxford, Oxford, United Kingdom.

The physiological environment of membrane proteins is a complex membrane yet structural studies by necessity are largely based on solubilisation in detergent micelles or model lipid bilayers and bicelles. The choice of solubilising environment has been shown to have some impact on the conformation of membrane proteins; understanding the basis of these changes is critical in linking experimental structures to the conformations expected in native environments.

Here we use a combination of coarse-grained (CG) and atomistic (AT) molecular dynamics (MD) simulations to probe the effects of experimental conditions on structure, with particular emphasis on the solution NMR structures of alpha helical membrane proteins including the influenza M2 proton channel and a mitochondrial carrier protein.

We show that this multiscale simulation approach may be used to improve the packing of solution NMR structures in a lipid bilayer environment. This technique is also used to probe the roles of specific native lipids (e.g. cardiolipin) on membrane protein structure and dynamics.

2097-Pos Board B116

On the Molecular Mechanism of Ion-Modulated Gating in Secondary Transporters

Sergei Noskov, Chunfeng Zhao, Bogdan Lev.
University of Calgary, Calgary, AB, Canada.

Ion-dependent secondary transporters are membrane proteins that couple electrochemical gradients to uphill substrate transport. The free energy of ion transport across the protein is converted into useful work to drive variety of cellular processes ranging from removal of neurotransmitter from synaptic cleft to delivery of key nutrients and osmolytes to the cell. The exact mechanism by which ion binding and transport is coupled to conformational dynamics of secondary transporters is yet to be established. It is generally believed that ion and/or substrate binding prepares and drives transporter into a certain conformational state along its transporting cycle. In this work, we have computed the multi-dimensional PMF profiles as function of ion/substrate occupancy for two different transporters (Mhp1 and Glt_{Ph}) representing major transporters super-fold families. The swarm-of-the trajectories string method has been used to construct minimum energy path connecting conformational states with known crystal structure. The role of the particular ion in the transport cycle was discussed and its coupling to the conformational dynamics of the gate unraveled. It was found that many of the ion-substrate load conformation display essentially barrier-less gating (HP2 gating in Glt_{Ph}) with binding of an ion and a substrate required to stabilize closed conformation of the gate. We have validated the results by comparison with experimentally measured gating dynamics of HP2 and biochemical evidence from studies of the conformational cycle of Mhp1 transporter.

2098-Pos Board B117

The Unusual Phenotype of the Mu Opioid Receptor S4.54A Mutant:An Exploration of Structural Origins using Conformational Memories

Elizabeth A. Pearsall[1], Ping-Yee Law[2], Patricia H. Reggio[1].
[1]University of NC at Greensboro, Greensboro, NC, USA, [2]University of Minnesota, Minneapolis, MN, USA.

A targeted gene therapy strategy has recently been reported that makes novel use of a μ-opioid receptor (MOR) S4.54A mutant at which the classical opioid antagonist, naloxone, becomes a partial agonist (Chen et. al, 2007, Kao et. al, 2010, Portoghese et. al, 2003). To probe the molecular origins of naloxone becoming a partial agonist in the S4.54A mutant, we use the Monte Carlo/simulated annealing technique, Conformational Memories (CM; Whitnell et. al, 2007) along with the recently published MOR crystal structure (Manglik, et. al, 2012) to study the effects of the mutant MOR on the shape of transmembrane helix 4 (TMH4) and the receptor as a whole. The MOR crystal structure shows that S4.54 faces lipid and is part of a small hydrogen bonding network which includes S3.30, Y3.34, S4.54 and G4.57. Here the lipid facing Y3.34 hydroxyl interacts with the polar sidechain of S4.54 and S3.30 forms a hydrogen bond with the backbone carbonyl of G4.57. Extracellular to the hydrogen bonding network is P4.59 in the MOR, which provides an extracelluar kink in TMH4. CM calculations revealed that the S4.54A MOR TMH4 straightens so that its extracellular end is closer to TMH5 rather than TMH3, as seen in the crystal structure. In addition, the proline kink in the S4.54A mutant (MT) is not as pronounced when compared with WT and the hydrogen bonding network between TMH3 and TMH4 is not intact in the S4.54A MT. using Glide (Schrodinger, 2011), we docked naloxone in both the WT MOR crystal structure and the S4.54A MT MOR structure. Results show that the receptor packs differently in the S4.54A MT such that naloxone sits higher in the binding pocket, thus allowing it to become a partial agonist.

2099-Pos Board B118

Rational Design of Neutral Allosteric Modulators of the CB1 Receptor with Improved Receptor Interactions and Unique Pharmacology

Derek M. Shore[1], Dow P. Hurst[1], Frank J. Navas[2], Herbert H. Seltzman[2], Gemma L. Baillie[3], Ruth A. Ross[3], Patricia H. Reggio[1].
[1]Center for Drug Discovery, UNC Greensboro, Greensboro, NC, USA, [2]Research Triangle Institute, Research Triangle Park, NC, USA, [3]Institute of Medical Sciences, University of Aberdeen, Aberdeen, United Kingdom.